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Antipseudomonal Activity of Simulated Infusions of Gentamicin Alone or with Piperacillin Assessed by Serum Bactericidal Rate and Area under the Killing Curve

JAMES E. TISDALE,[1]† MARY T. PASKO,[1,2] AND JOSEPH M. MYLOTTE[2,3,4]*

School of Pharmacy[1] and Departments of Medicine[4] and Microbiology,[3] State University of New York at Buffalo, and Veterans Administration Medical Center,[2] Buffalo, New York 14215*

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The objectives of this study were to (i) determine which of three simulated dosing regimens (gentamicin alone, simultaneous infusions of gentamicin and piperacillin, or staggered infusions of gentamicin and piperacillin) produced the fastest killing rate of *Pseudomonas aeruginosa* in serum, using the serum bactericidal rate (SBR) assay; and (ii) describe an alternative method of analysis of killing curves, the area under the killing curve (AUKC). Gentamicin alone or combined with piperacillin was added to heat-inactivated human serum to approximate drug concentrations achieved after the above-mentioned types of infusion. By a microdilution technique, seven strains of *P. aeruginosa* were exposed to no drug (control) and gentamicin alone or with piperacillin; colony counts were determined at hourly intervals for 5 h, and $\log_{10}$ CFU per milliliter was plotted versus time. Linear regression was used to calculate the slope (SBR) of each timed killing curve for each drug concentration tested alone or in combination. In addition, the AUKC for each curve was calculated. To compare simulated infusion regimens further, the cumulative AUKC (the sum of AUKCs for specific time points along the serum concentration-time curve for each simulated regimen) was calculated. With the SBR assay or AUKC determination, there was a significant increase in the rate of killing of all test strains by the combination compared with gentamicin alone only at gentamicin concentrations which exceeded the MIC (8, 5, and 2.5 μg/ml). Mean cumulative AUKC of the simultaneous-infusion regimen was significantly less (indicating faster killing) than either the staggered-infusion regimen or the gentamicin infusion alone. Both the SBR and AUKC have the potential for integration of in vitro microbiologic effects and in vivo pharmacokinetics of antimicrobial agents.

Recently, the rate of serum bacterial killing, or serum bactericidal rate (SBR), has been investigated as a measure of the antimicrobial activity of antibiotics alone and in combination (5, 7, 26, 29, 33–35). The SBR assay, first described by Drake et al. (7), is an abbreviated timed killing curve performed in serum, rather than broth. Unlike the timed killing curve method (18) and the checkerboard technique (2), which are performed in broth medium, the SBR assay allows consideration of both pharmacokinetics and serum protein binding of the antimicrobial agent(s) under study (5). Briceland et al. (5) modified the SBR assay of Drake et al. (7) in such a way as to quantitate the rate of killing; Mordenti et al. (26) have published a similar method. The latter approach allows for statistical comparison of rates of killing of antimicrobial agents rather than the application of arbitrary criteria for such comparisons.

The purpose of the present study was to expand our previous work with the SBR assay (5), which suggested significant enhancement of the SBR of gentamicin by the addition of piperacillin for three gentamicin-susceptible strains of *Pseudomonas aeruginosa* only at gentamicin concentrations below the MIC for the test strains. To test this finding further, the present study was developed to measure the SBR of gentamicin, with and without piperacillin, for multiple strains of *P. aeruginosa* at drug concentrations chosen to simulate three infusion regimens: administration of gentamicin alone, simultaneous administration of gentamicin and piperacillin, and staggered administration such that the peak serum piperacillin concentration occurs as the serum gentamicin concentration approaches or falls below the MIC for a particular isolate.

In addition, we describe an alternative approach for evaluating timed killing curves which consisted of measurement of the area under the killing curve (AUKC). A method of applying AUKC measurements for comparison of the simulated-infusion regimens noted above is also described.

MATERIALS AND METHODS

Test bacteria. Seven strains of *P. aeruginosa* were obtained from the Microbiology Laboratory of the Veterans Administration Medical Center, Buffalo, N.Y. Organisms were isolated from blood (three strains) or sputum (four strains). Each strain was identified as *P. aeruginosa* by standard methods. The strains were maintained on Mueller-Hinton agar and subcultured weekly.

Donor serum. Serum was obtained from several healthy volunteers who were taking no medication. The serum was inactivated by heating to 56°C for 30 min to eliminate the possible factor of intrinsic bactericidal activity against the test strains. It was kept frozen at −20°C until used.

Antibiotics. Antibiotics were kindly provided by their respective manufacturers: Schering Corp., Kenilworth, N.J. (gentamicin); and Lederle Laboratories, Pearl River, N.Y. (piperacillin). The antibiotic concentrations selected for testing were chosen such that they approximated those achieved in serum after the intravenous administration of gentamicin (2 mg/kg) alone, gentamicin (2 mg/kg) and piperacillin (4 g) simultaneously, or gentamicin (2 mg/kg) followed 3.5 h later

* Corresponding author.
† Present address: Drug Information Center, Hartford Hospital, Hartford, CT 06115-0729.

TABLE 1. Concentrations in serum of gentamicin and piperacillin used to simulate three infusion regimens

Infusion regimen	Concn (μg/ml) at given time after completion of simulated gentamicin infusion[a]				
	0.5 h	2.5 h	4.0 h	5.0 h	5.5 h
Gentamicin (2 mg/kg)	8.0	5.0	2.5	1.5	1.0
Simultaneous infusion of gentamicin (2 mg/kg) and piperacillin (4 g)	8.0/250	5.0/100	2.5/25	1.5/10	1.0
Infusion of gentamicin (2 mg/kg) followed 3.5 h later by piperacillin (4 g)	8.0	5.0	2.5/250	1.5/50	1.0/25

[a] One value in a column represents a serum gentamicin concentration; when two values are given, the first represents a serum gentamicin concentration and the second represents a serum piperacillin concentration.

by an infusion of piperacillin (4 g) (1, 30). The serum antibiotic concentrations chosen to simulate these three regimens are listed in Table 1.

Gentamicin and piperacillin stock solutions were prepared with donor serum and were kept frozen at −20°C until used. These combinations of gentamicin and piperacillin were prepared just prior to use. Stock solutions were assayed prior to use to verify the concentrations present.

MIC determination. MICs of gentamicin and piperacillin for the *P. aeruginosa* test strains were determined by using an automated system (Vitek Systems, Hazelwood, Mo.). The inoculum for this system was prepared by growing a test strain in Mueller-Hinton broth to a turbidity equivalent to 0.5 McFarland standard at 600 nm. This was further diluted 1:36 in sterile saline, and this dilution was distributed on the Vitek plates containing growth media by using an automated delivery system. This produced a final inoculum of 10^5 to 10^6 CFU/ml.

Experimental design. Each gentamicin concentration was tested alone and in combination with various concentrations of piperacillin (Table 1) for a given test strain of *P. aeruginosa*. Each experiment was performed with seven test samples of 0.2 ml: heat-inactivated serum only (control), three serum samples each containing a different concentration of gentamicin alone, and three serum samples containing gentamicin at the concentrations tested alone plus piperacillin in concentrations intended to simulate one of the clinical situations described previously. Mueller-Hinton broth (0.01 ml) containing a test strain was added to each test sample.

Timed killing curve method. A microdilution technique was used in this study in contrast to our previous study (5), in which a macrodilution technique was used. A test strain of *P. aeruginosa* was grown overnight in Mueller-Hinton broth at 37°C. The optical density of the broth was adjusted to 0.135 at 660 nm and diluted 1:50 in sterile phosphate-buffered saline. A 0.01-ml portion of the 1:50 dilution was added to 0.2 ml of test serum, providing a final inoculum of approximately 10^5 to 10^6 CFU/ml. Test samples were incubated at 37°C without shaking. At 0, 1, 2, 3, 4, and 5 h after inoculation, colony counts of bacteria in each test sample were determined in the following manner. A 0.01-ml portion was removed from each sample and serially diluted in sterile phosphate-buffered saline; 0.05 ml of each dilution was subcultured onto Mueller-Hinton agar with a spiral plater (Spiral Systems Inc., Cincinnati, Ohio). Colony counts were determined after 18 h of incubation at 37°C. The minimal CFU per milliliter detectable by this method was 200. For each serum sample tested, the $\log_{10}$ CFU of viable bacteria remaining per milliliter was plotted versus time of incubation.

Two methods were used to minimize and account for antibiotic carryover on subculture plates. From time zero to 3 h of incubation, the majority of samples were diluted 100- to 10,000-fold (20). At 4 and 5 h of incubation, particularly with higher concentrations of antibiotic studied, colony counts decreased to a level at which such high dilutions (≥1,000-fold) could not be used in some experiments. In this situation, a 0.05-ml portion was removed from a microdilution well and subcultured directly onto an agar plate without dilution; 10- and 100-fold dilutions of a separate 0.05-ml sample were also made, and 0.05 ml of each dilution was subcultured. The colony count resulting from subculture of an undiluted portion of a test sample was compared with that resulting from subcultures of the dilutions of the same test sample. When the concentration of bacteria calculated by using colony counts in undiluted subculture samples was less than that calculated by using results from the dilutions, possibly representing the effects of antibiotic carryover, the bacterial concentration was calculated by using the results of the colony counts in the 10- and 100-fold dilutions. Also, antibiotic carryover was further minimized for all undiluted test samples and 10-fold-diluted test samples by subculturing on Mueller-Hinton agar supplemented with polyanetholesulfonic acid (19) at a concentration of 2.5 mg/ml of agar for the inactivation of gentamicin or supplemented with polyanetholesulfonic acid and β-lactamase for the inactivation of gentamicin-piperacillin combinations.

Statistical analysis and SBR calculation. By using the statistical Package for the Social Sciences, multivariate repeated-measures analysis of variance (ANOVA) with polynomial transformation (4, 11) was performed. This analysis evaluated the trend for each curve (linear, quadratic, cubic, or quartic) which best described the relationship between $\log_{10}$ CFU per milliliter and time of exposure to one or both agents. Through the univariate results, the strongest trend was determined and found to be the linear component. In some cases, there were weak quadratic or cubic trends, but the linear was the predominant and overriding relationship. Based on this analysis and the previous study (5), linear regression analysis was used to determine the SBR. The SBR was defined as the slope of the regression line, and its units were the change in the $\log_{10}$ CFU per milliliter per hour of exposure to the agent(s). The more negative the slope, the faster the rate of bacterial killing. There was always a positive slope, indicating growth, in serum without antibiotic (control) for all strains tested. The slope of the control samples ranged from +0.03 to +0.22 (mean, +0.14). Repeated-measures ANOVA was used to compare mean SBRs of gentamicin alone or in combination with piperacillin. Student's *t* test for paired data (6) with the Bonferroni adjustment (16, 22) was used to test significant differences.

As an alternative method of measuring bacterial killing, AUKC was calculated for each drug concentration or combination. This was done by plotting the specific colony counts at each time point of an individual experiment and determining the AUKC by using the trapezoidal rule (12). In this type of analysis the rate of killing is inversely related to the AUKC; i.e., the faster the rate of killing, the smaller the AUKC. To compare the overall effects of the three simulated infusion regimens on the rate of killing of *P. aeruginosa*, the cumulative AUKC for each regimen was calcu-

lated. The cumulative AUKC is defined as the sum of the AUKCs calculated for each of the five time points along the serum concentration-time curve for each regimen (Table 1). There were three cumulative AUKC determinations (one for each of the three simulated regimens) per test strain. Repeated-measures ANOVA and Student's t test were used to compare mean AUKCs. For all statistical analysis, $P \leq 0.05$ was considered significant except when Bonferroni adjustment was applied to maintain an overall level of significance of 0.05 (16).

Antibiotic assay. The gentamicin concentration in stock solutions was determined by using a fluorescence polarization immunoassay technique on the Optimate system (Ames Division, Miles Laboratories, Inc., Elkhart, Ind.) in the Clinical Chemistry Laboratory at the Veterans Administration Medical Center, Buffalo, N.Y. The coefficients of variation for the assay are 4.5% at 2 μg of gentamicin per ml and 3.1% at 10 μg of gentamicin per ml. The sensitivity of this assay is 1.0 μg/ml. The piperacillin concentration in the stock solutions was determined by using an agar well diffusion assay (9). During several experiments, serum containing both gentamicin (at 8, 5, and 2.5 μg/ml only) and piperacillin (at 250 and 100 μg/ml only) was assayed at time zero and at the end of a 5-h incubation period. There was no difference in the gentamicin concentration at these times, indicating that there was no inactivation of gentamicin by piperacillin during the incubation period.

RESULTS

Microbiologic activity. All seven test strains of *P. aeruginosa* were susceptible to gentamicin and piperacillin. The gentamicin MIC ranged from 1 to 2 μg/ml for six strains, and it was <0.5 μg/ml for the remaining isolate. Piperacillin MICs were ≤8 μg/ml for all seven test strains.

Analysis of curve trends. Multivariate repeated-measures ANOVA with polynomial transformation was performed on pooled data from experiments with all seven test strains for each concentration of gentamicin alone or concentration pairs (Table 1). All curves for gentamicin alone at concentrations above the MIC showed a significant linear trend ($P < 0.008$), as did all combinations tested (P values ranging from 0.03 to 0.0001). Cubic and quartic trends also described several curves; however, the trends were predominately linear for all but gentamicin at 2.5 and 1.0 μg/ml.

SBR of gentamicin alone and gentamicin plus piperacillin. The mean SBRs of gentamicin alone and of gentamicin plus piperacillin in various combinations for the seven test strains of *P. aeruginosa* are shown in Table 2. Concentration-dependent killing was seen at gentamicin concentrations that exceeded the MIC (8 > 5 > 2.5 μg/ml; $P < 0.0009$). The mean SBRs for gentamicin at 2.5 and 1.5 μg/ml were not significantly different ($P = 0.0367$; Bonferroni adjustment, $P = 0.0125$). For gentamicin concentrations of 8 and 5 μg/ml, the addition of piperacillin significantly increased the SBR in comparison with gentamicin alone. When the gentamicin concentration was reduced to 2.5 μg/ml (slightly above the MIC for most test strains), there was a significant difference between the SBRs of gentamicin alone and in combination with 250 μg of piperacillin per ml. For gentamicin concentrations of 1.5 and 1.0 μg/ml, the addition of piperacillin did not significantly affect the rate of killing in comparison with gentamicin alone.

AUKC of gentamicin alone and gentamicin plus piperacillin. Table 3 contains the mean AUKC data for gentamicin alone and gentamicin combined with piperacillin. The addition of piperacillin to gentamicin at concentrations of 8, 5, and 2.5 μg/ml significantly decreased the calculated AUKC compared with gentamicin alone, indicating more rapid killing with the drug combination. At gentamicin concentrations below the MIC (1.5 and 1.0 μg/ml) for the test strains, the addition of piperacillin did not significantly change the mean AUKC compared with that of gentamicin alone.

Similar to the findings with the SBR assay, the killing rate of gentamicin alone as measured by the mean AUKC was concentration dependent for drug concentrations above the MIC (8 > 5 > 2.5 μg/ml) (ANOVA; $P = 0.002$). However, the mean AUKCs for gentamicin at 8 (21.68 ± 1.76) and 5 (24.29 ± 1.90) μg/ml, although numerically indicating a greater killing for gentamicin at 8 μg/ml, were not significantly different ($P = 0.025$) when the Bonferroni adjustment ($P = 0.01$) was applied.

TABLE 2. Mean SBRs of gentamicin and gentamicin-piperacillin combinations

Antibiotic concn (μg/ml)[a]	Mean SBR ± SD[b]	P
8/0	−0.62 ± 0.12	0.038
8/250	−0.84 ± 0.33	
5/0	−0.43 ± 0.09	0.016
5/100	−0.63 ± 0.22	
2.5/0	−0.12 ± 0.16[c]	
2.5/25	−0.47 ± 0.14[c]	0.001[d]
2.5/250	−0.51 ± 0.12[c]	
1.5/0	−0.26 ± 0.13	
1.5/10	−0.36 ± 0.17	0.102[d]
1.5/50	−0.37 ± 0.12	
1.0/0	−0.08 ± 0.12	0.59
1.0/25	−0.14 ± 0.06	

[a] Values given are gentamicin and piperacillin concentrations, respectively.
[b] Change in $\log_{10}$ CFU per milliliter per hour of antibiotic exposure. There were seven SBR determinations per concentration(s) tested.
[c] Paired t test: 2.5/0 versus 2.5/25, $P = 0.25$; 2.5/0 versus 2.5/250, $P = 0.004$; 2.5/25 versus 2.5/250, $P = 0.17$. Bonferroni's adjustment for significance was $P = 0.0167$.
[d] ANOVA.

TABLE 3. Mean AUKC for gentamicin alone and in combination with piperacillin

Concn (μg/ml) of gentamicin-piperacillin	Mean AUKC ± SD[a]	P
8/0	21.68 ± 1.76	0.005
8/250	17.18 ± 2.63	
5/0	24.29 ± 1.90	0.002
5/100	20.98 ± 1.09	
2.5/0	28.80 ± 2.13[b]	
2.5/25	24.25 ± 1.79[b]	0.0002
2.5/250	23.06 ± 2.28	
1.5/0	27.01 ± 1.96	0.238
1.5/10	26.46 ± 2.14	
1.5/50	26.12 ± 1.83	
1.0/0	28.37 ± 0.93	0.07
1.0/25	29.17 ± 1.26	

[a] There were seven AUKC determinations per concentration(s) tested.
[b] $P = 0.0007$.

TABLE 4. Cumulative AUKCs for simulated gentamicin and piperacillin administration

Organism	AUKC[a]		
	Gentamicin alone	Gentamicin-piperacillin simultaneously	Gentamicin-piperacillin staggered[b]
101PS2	124.77	108.16	113.51
85-3575	128.32	119.46	128.80
85-2733	134.71	121.64	126.31
16990	120.28	109.93	118.30
14676-2	137.80	126.31	134.51
1519-2	124.47	113.38	122.62
1704	126.65	120.33	123.60
Mean ± SD	128.14 ± 6.13[c]	117.03 ± 6.67[d]	123.97 ± 6.87[e]

[a] ANOVA, $P = 0.0001$. Paired t test (Bonferroni adjustment, $P = 0.0167$): c versus d, $P = 0.0001$; d versus e, $P = 0.0001$; c versus e, $P = 0.036$.
[b] Infusions were separated by 3.5 h.

Comparison of cumulative AUKCs of simulated infusion regimens. The results of the serum killing rates of gentamicin alone and with piperacillin, using the SBR assay (Table 2) and AUKC analysis (Table 3), suggested that there may be an advantage for the simultaneous administration of gentamicin and piperacillin compared with either gentamicin alone or the staggered regimen. To test this hypothesis, the cumulative AUKC for each simulated infusion regimen for each test strain was calculated (Table 4). The mean cumulative AUKC value for the simultaneous regimen was significantly less than that for the staggered regimen or gentamicin alone (ANOVA; $P = 0.0001$). Since the rate of killing is inversely related to the magnitude of the AUKC, the simulated simultaneous regimen produced the fastest killing of the test strains over the interval studied. The mean cumulative AUKC for the staggered regimen was not different from that of the gentamicin infusion alone ($P = 0.036$; Bonferroni adjustment, $P = 0.0167$).

Test variability. The mean interday variability for SBR determinations for the same drug concentrations and test strains was 24.6%. The mean interday variability of the AUKC calculations was 9.8%.

DISCUSSION

The bactericidal activity of antimicrobial combinations has been evaluated by using a number of different in vitro methods, most commonly the timed killing curve method (18) and the checkerboard technique (2). Although these assays have been shown to be useful (10), they have several drawbacks. They are performed in broth, eliminating the factor of serum protein binding from consideration. Arbitrarily chosen antibiotic concentrations are often tested and may not necessarily be representative of those achieved in vivo. Criteria for evaluation of results of these tests are often chosen arbitrarily, particularly for the timed killing curve method. Although these tests provide a method for the evaluation of the pharmacodynamic interaction between antibiotic and microorganism, they do not take into consideration drug pharmacokinetics.

Recently, the rate of serum bacterial killing has been used to measure the antimicrobial activity of serum from volunteers given an antibiotic (33–35). However, at the present time, there is no information regarding the relationship between the magnitude of the serum bactericidal rate and the efficacy of an antibiotic regimen in humans. Drake et al. (7) compared killing rates of nafcillin alone and with an aminoglycoside in a rabbit model of *Staphylococcus aureus* endocarditis. In this study, in vitro tests were performed with rabbit serum to simulate more closely the in vivo situation. These investigators found a significantly more rapid in vitro serum killing rate of the test strain of *S. aureus* by nafcillin plus gentamicin compared with nafcillin alone. This correlated directly with the in vivo findings of a greater reduction in colony counts of *S. aureus* in valvular vegetations with combination therapy versus nafcillin alone. Therefore, this study provides evidence that, at least for this animal model, detectable differences in in vitro killing rates in serum of various regimens correlated well with differences in antibacterial effects at the tissue site of infection.

For the seven strains of *P. aeruginosa* tested, the addition of piperacillin to gentamicin concentrations which exceeded the MIC (8, 5, and 2.5 μg/ml) significantly enhanced the killing rate. In our previous study (5), although the magnitude of the SBRs of the combination was greater than that of gentamicin alone, it did not become statistically significant until the gentamicin concentration was below the MIC. One explanation for the difference may be the larger number of test strains (seven) utilized in the present study versus the number (three) studied previously (5). Also, it should be noted that two changes in method were made compared with the previous work (5). The SBR assay performed by Briceland et al. (5) was conducted with a macrodilution technique, whereas the present work was conducted with a microdilution technique, which allowed the use of much smaller quantities of serum.

The second methodologic change in the present study was a reduction in the duration of incubation of test samples from 8 to 5 h. Previous work with timed kill curves has been usually carried out for a period of 18 to 24 h (18). Drake et al. (7) arbitrarily reduced the incubation period to 8 h, and this same 8-h incubation period was used in our previous study of the SBR. However, observations made during that study (5) revealed a phase of rapid killing of *P. aeruginosa* during the first 5 h of drug exposure with gentamicin alone or gentamicin plus piperacillin. This rapid killing phase was followed by a lower rate of killing during the last 3 h of incubation. In the present study, we chose to evaluate only the phase of rapid killing; therefore, the incubation period was 5 h long. We also demonstrated that this reduction in incubation time to 5 h enhanced the validity of using linear regression to analyze the killing curves.

It should be kept in mind that in vitro tests intended to evaluate the interaction of bacteria and antibiotics may grossly overestimate the actual exposure time of the microorganism to a fixed drug concentration. To simulate more closely the in vivo situation, it would be necessary to devise a mechanism that would allow the exposure of an organism to changing drug concentrations over time. Such systems have been devised by several groups of investigators (17, 27, 31, 36). However, all of these systems utilize broth as a diluent rather than serum. In interpreting the results of studies done with these systems, two factors should be kept in mind. First, the rate of killing of β-lactams and cephalosporins depends strictly on the bacterial growth rate (32). Second, the rate of bacterial growth in serum tends to be slower than in broth media (J. M. Mylotte and E. Stewart, unpublished data). Thus, one must be cautious in not overinterpreting the results of studies done in broth media. In addition, a recent study (21) has suggested that there is an unidentified factor(s) in serum which appears to enhance the activity of antimicrobial agents against members of the family *Enterobacteriaceae* (but not *P. aeruginosa*). Again,

such a phenomenon is not present when in vitro tests are done in broth media only.

The abbreviated time-kill experiments used in the present study will not detect gentamicin-resistant subpopulations of *P. aeruginosa* (13). The importance of this phenomenon in the regrowth of bacteria after exposure to aminoglycoside alone has been noted both in vitro (3, 14) and in vivo (13–15). The SBR assay and AUKC as described in this study deal only with the initial 5 h of drug-bacterium interaction. The regrowth of aminoglycoside-resistant subpopulations usually does not occur until later (beyond 8 h) in the drug-bacterium interaction. Such resistant populations can be suppressed by the antipseudomonal penicillins when used in combination with the aminoglycoside (15) or by high levels (8- to 16-fold greater than the MIC) of aminoglycoside (28). It should be kept in mind that the SBR assay was meant to describe the bacterial killing rate at one point in time during a dosing interval. This approach provides a method for statistical comparison of the early phase of bacterial killing by antibiotics alone and in combination.

In the present report, an alternative method, AUKC, for quantitating the rate of serum killing of bacteria by antimicrobial agents is described. The results of applying this method to the analysis of killing curves generated in this study (Table 3) were consistent with the findings with the SBR assay (Table 2). Specifically, with both methods, the addition of piperacillin to concentrations of gentamicin above the MIC for the test strains significantly increased the rate of killing. However, the interday variability of these methods was considerably different. This difference in variability may primarily be related to differences in methods of data analysis. That is, the SBR is at best an approximation (albeit a statistically valid one) of the rate of bacterial killing as determined by linear regression analysis; the AUKC, on the other hand, takes into consideration or allows for "nonlinear" bends in the curves.

Thus, determination of the AUKC may be a more accurate and reproducible method of evaluating antibiotic interactions than the SBR assay as defined in this study and previously (5). The AUKC determination is directly and easily calculated from the "raw" data generated from time killing curves, can be statistically compared as demonstrated in the present study, and has an acceptable degree of day-to-day variability. Nevertheless, both assays have detected an increasing rate of killing by gentamicin alone with increasing concentration, i.e. concentration-dependent killing, which has been described previously (6, 14). This finding coupled with recent studies (23–25) identifying a relationship between peak serum aminoglycoside concentration or the ratio of peak concentration to MIC and outcome in patients with gram-negative aerobic pneumonia or bacteremia suggests that the SBR assay or AUKC analysis may be clinically useful.

Although further study is required to determine the role of the assays in evaluating the clinical use of antimicrobial agents alone and in combination, the integration of antibiotic pharmacokinetics and pharmacodynamics in the treatment of infections is becoming increasingly important (8). We would not advocate the use of the SBR or the AUKC analysis in all situations in which antimicrobial combinations are being administered. However, these methods may be useful in evaluating the efficacy of antimicrobial combinations in patients in whom the rate of bacterial killing may be important, such as those with gram-negative pneumonia or bacterial meningitis, and in the granulocytopenic patient with gram-negative bacteremia. Also, these approaches may be useful for evaluating experimental agents.

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